

ET



**PCT**

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> : <b>A61K 31/405, 38/06, 38/07</b></p>	<p><b>A1</b></p>	<p>(11) International Publication Number: <b>WO 99/66930</b> (43) International Publication Date: 29 December 1999 (29.12.99)</p>
<p>(21) International Application Number: PCT/US99/13888 (22) International Filing Date: 21 June 1999 (21.06.99) (30) Priority Data: 60/090,404 24 June 1998 (24.06.98) US 9817174.7 6 August 1998 (06.08.98) GB (71) Applicant (for all designated States except US): MERCK &amp; CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): RESZKA, Alfred, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (74) Common Representative: MERCK &amp; CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).</p>		<p>(81) Designated States: AE, AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: COMPOSITIONS AND METHODS FOR TREATING ELEVATED BLOOD CHOLESTEROL</p> <p>(57) Abstract</p> <p>The present invention relates to compositions and methods for treating elevated blood cholesterol in a mammal while counteracting the occurrence of potentially adverse side effects such as myopathy. The compositions useful herein comprise the combination of a pharmaceutically effective amount of a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (hereafter "HMG-CoA reductase inhibitor") and a caspase inhibitor to a mammal in need thereof.</p>		

BEST AVAILABLE COPY

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

TITLE OF THE INVENTION  
COMPOSITIONS AND METHODS FOR TREATING ELEVATED BLOOD  
CHOLESTEROL

5 BRIEF DESCRIPTION OF THE INVENTION

The present invention relates to compositions and methods for treating elevated blood cholesterol in a mammal while counteracting potential adverse side effects such as myopathy. The compositions useful herein comprise the combination of a pharmaceutically effective amount of a 3-hydroxy-3-methylglutaryl coenzyme A  
10 reductase inhibitor (hereafter "HMG-CoA reductase inhibitor") and a caspase inhibitor to a mammal in need thereof.

BACKGROUND OF THE INVENTION

It has been clear for several decades that elevated blood cholesterol is a  
15 major risk factor for coronary heart disease, and many studies have shown that the risk of coronary heart disease (CHD) events can be reduced by lipid-lowering therapy. Prior to 1987, the lipid-lowering armamentarium was limited essentially to a low saturated fat and cholesterol diet, the bile acid sequestrants (cholestyramine and colestipol), nicotinic acid (niacin), the fibrates and probucol. Unfortunately, all of  
20 these treatments have limited efficacy or tolerability, or both. Substantial reductions in LDL (low density lipoprotein) cholesterol accompanied by increases in HDL (high density lipoprotein) cholesterol could be achieved by the combination of a lipid-lowering diet and a bile acid sequestrant, with or without the addition of nicotinic acid. However, this therapy is not easy to administer or tolerate and was therefore often  
25 unsuccessful except in specialist lipid clinics. The fibrates produce a moderate reduction in LDL cholesterol accompanied by increased HDL cholesterol and a substantial reduction in triglycerides, and because they are well tolerated these drugs have been more widely used. Probuco produces only a small reduction in LDL cholesterol and also reduces HDL cholesterol, which, because of the strong inverse  
30 relationship between HDL cholesterol level and CHD risk, is generally considered undesirable. With the introduction of lovastatin, the first inhibitor of HMG-CoA reductase to become available for prescription in 1987, for the first time physicians were able to obtain large reductions in plasma cholesterol with very few adverse effects.

Recent studies have unequivocally demonstrated that lovastatin, simvastatin and pravastatin, all members of the HMG-CoA reductase inhibitor class, slow the progression of atherosclerotic lesions in the coronary and carotid arteries. Simvastatin and pravastatin have also been shown to reduce the risk of coronary heart disease events, and in the case of simvastatin a highly significant reduction in the risk of coronary death and total mortality has been shown by the Scandinavian Simvastatin Survival Study. This study also provided some evidence for a reduction in cerebrovascular events.

However, along with their benefits, HMG-CoA reductase inhibitors can cause potentially adverse side effects such as myopathy and related disorders in a small percentage of patients. Myopathy is characterized by muscle pain and weakness. The Physician's Desk Reference, 42nd Ed., 1366 (1988), which is incorporated by reference herein in its entirety, states that myalgia, i.e. muscle pain, has been associated with lovastatin. Tobert, *N.E.J.Med.*, 48 (January 7, 1988), which is incorporated by reference herein in its entirety, states that in a very small number of patients (0.5 percent) myopathy appeared to be associated with lovastatin therapy. Concomitant therapy with immunosuppressant drugs, including cyclosporine, with gemfibrozil, or niacin, or a combination, appears to increase the risk of myopathy. See, J.A. Tobert, *Am.J. Cardiol.*, 1988, 62: 28J-34J, which is incorporated by reference herein in its entirety. The myopathy is reversible upon discontinuation of lovastatin therapy. See U.S. Patent 4, 933, 165, to Brown, issued June 12, 1990, which is incorporated by reference herein in its entirety. It is seen that it would be of considerable benefit to counteract the myopathy observed in the small percentage of patients. Therefore, improved therapies for treating, preventing, and reducing the risk of developing atherosclerosis, and cardiovascular and cerebrovascular events and related disorders are currently being sought which minimize the potential for adverse effects such as myopathy.

Caspases belong to a broad class of enzymes known as proteases, which are enzymes that hydrolyze peptide bonds. Specifically, caspases are cysteine proteases that preferentially target and cleave peptide sequences having an aspartic acid moiety. Caspases are believed to be involved in the normal cell turnover process and are mediators of apoptosis. See Nicholson and Thornberry, 1997, "Caspases: killer proteases", *TIBS*, vol. 22, 299-306; Henkart, 1996, "ICE family proteases: mediators of all apoptotic cell death?" *Immunity*, 4, 195-201; Ray et al, 1992 "Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1 beta

converting enzyme", *Cell* , 69, 596-604; Enari et al, 1995, "Involvement of an ICE-like protease in fas-mediated apoptosis", *Nature* I, 375, 78-81; Enari et al, 1996, "Sequential activation of ICE-like and CPP32-like proteases during Fas-mediated apoptosis", *Nature* , 380, 723-726; Los et al, 1995, "Requirement of an ICE/CED-3 protease for Fas/APO-1 mediated apoptosis", *Nature* , 375, 81-83; and Tewari et al., 1995, "Fas and tumor necrosis factor-induced apoptosis is inhibited by the poxvirus crmA gene product", *J. Biol. Cheml.* , 270, 3255-3260; which are all incorporated by reference herein in their entirety.

It is found in the present invention that caspase inhibitors can block the apoptosis, i.e. programmed cell death, that can be induced by HMG-CoA reductase inhibitors. However, caspase inhibitors have not previously been investigated either *in vitro* or *in vivo* for their ability to mitigate the potentially adverse myopathy side effects that can be associated with HMG-CoA reductase inhibitor therapy for treating or preventing elevated blood cholesterol.

In the present invention, it is found that the combination of an HMG-CoA reductase inhibitor and a caspase inhibitor is effective for treating or preventing elevated blood cholesterol while mitigating the potentially adverse myopathy side effects that can be associated with the therapy. The combination has the advantage of providing increased safety and better patient compliance, which should maximize therapeutic efficacy. Without being limited by theory it is believed that the caspase inhibitor blocks the potentially harmful effect of the HMG-CoA reductase inhibitor on muscle cells. In other words, the caspase inhibitor is believed to interfere with apoptosis which can potentially be induced in muscle cells by the HMG-CoA reductase inhibitor.

It is an object of the present invention to provide compositions comprising the combination of an HMG-CoA reductase inhibitor and a caspase inhibitor.

It is another object of the present invention to provide methods for treating or preventing elevated blood cholesterol in a mammal, particularly wherein said mammal is a human.

It is another object of the present invention to provide such methods while counteracting potential adverse myopathy effects.

It is another object of the present invention to provide such methods wherein the dosing is maintained until the desired therapeutic effect is achieved and/or maintained.

These and other objects will become readily apparent from the detailed description which follows.

#### SUMMARY OF THE INVENTION

5           The present invention relates to a pharmaceutical composition comprising an HMG-CoA reductase inhibitor and a caspase inhibitor.

          In further embodiments the present invention relates to a pharmaceutical composition comprising a pharmaceutically-effective amount of an HMG-CoA reductase inhibitor and an amount of a caspase inhibitor effective to  
10       counteract HMG-CoA reductase-associated myopathy.

          In further embodiments, the present invention relates to a method for treating or preventing elevated blood cholesterol in a mammal in need thereof comprising administering an HMG-CoA reductase inhibitor and a caspase inhibitor.

          In further embodiments, the present invention relates to a method for  
15       treating or preventing elevated blood cholesterol in a mammal in need thereof comprising sequentially administering a caspase inhibitor and an HMG-CoA reductase inhibitor.

          In further embodiments, the present invention relates to the use of a composition in the manufacture of a medicament for treating or preventing elevated  
20       blood cholesterol in a mammal in need thereof, said composition comprising an HMG-CoA reductase inhibitor and a caspase inhibitor.

          In further embodiments, the present invention relates to the use of a composition comprising an HMG-CoA reductase inhibitor and a caspase inhibitor for treating or preventing elevated blood cholesterol in a mammal in need thereof.

25           All percentages and ratios used herein, unless otherwise indicated, are by weight. The invention hereof can comprise, consist of, or consist essentially of the essential as well as optional ingredients, components, and methods described herein.

#### BRIEF DESCRIPTION OF THE FIGURE

30           Figure 1 shows that activation of Mst1 cleavage by 10  $\mu$ M lovastatin is blocked by the caspase inhibitors Z-Asp-Glu-Val-Asp-FMK, Z-Val-Ala-Asp-FMK, or Z-Tyr-Val-Ala-Asp-FMK. Osteoclast like cells are purified from cocultures by sequential treatment of culture dishes with collagenase and EDTA. Cells are then treated for 17 hours with lovastatin, and cell lysates are analyzed by an in-gel kinase  
35       assay using myelin basic protein as a substrate. Lane 1 is a no-treatment control. Lane

2 shows treatment with 20  $\mu$ M Z-Asp-Glu-Val-Asp-FMK. Lane 3 shows treatment with 20  $\mu$ M Z-Val-Ala-Asp-FMK. Lane 4 shows treatment with 20  $\mu$ M Z-Tyr-Val-Ala-Asp-FMK. Lane 5 shows treatment with 10  $\mu$ M lovastatin. Lane 6 shows treatment with the combination of 10  $\mu$ M lovastatin and 20  $\mu$ M Z-Asp-Glu-Val-Asp-FMK. Lane 7 shows treatment with the combination of 10  $\mu$ M lovastatin and 20  $\mu$ M Z-Val-Ala-Asp-FMK. Lane 8 shows treatment with the combination of 10  $\mu$ M lovastatin and 20  $\mu$ M Z-Tyr-Val-Ala-Asp-FMK.

#### DETAILED DESCRIPTION OF THE INVENTION

10           The present invention relates to compositions and methods for treating or preventing elevated blood cholesterol in a mammal in need of such treatment, while counteracting the occurrence of adverse myopathy effects. The compositions comprise a pharmaceutically effective amount of an HMG-CoA reductase inhibitor and a pharmaceutically effective amount of a caspase inhibitor.

15           The term "pharmaceutically effective amount", as used herein, means that amount of the HMG-CoA reductase inhibitor or caspase inhibitor that will elicit the desired therapeutic effect or response or provide the desired benefit when administered in accordance with the desired treatment regimen. A preferred pharmaceutically effective amount of the HMG-CoA reductase inhibitor is an amount  
20 that is effective for treating or preventing elevated blood cholesterol. A preferred pharmaceutically effective amount of the caspase inhibitor is an amount that will block or mitigate the occurrence of adverse myopathy effects, while not blocking, or only minimally blocking, the therapeutic blood cholesterol effects of the HMG-CoA reductase inhibitor.

25           The term "counteracting the occurrence of adverse myopathy effects", as used herein, means preventing, decreasing, or lessening the occurrence of unwanted muscular effects, relative to treatment with a HMG-CoA reductase inhibitor alone.

          The term "until the desired therapeutic effect is achieved and/or maintained", as used herein, means that the therapeutic agent or agents are  
30 continuously administered, according to the dosing schedule chosen, up to the time that the clinical or medical effect sought for the disease or condition being treated is observed by the clinician or researcher. For methods of treatment of the present invention, the pharmaceutical composition is continuously administered until the desired change in blood cholesterol is observed. In such instances, achieving a

decrease in blood cholesterol is a desired objective. For methods of prevention of the present invention, the pharmaceutical composition is continuously administered for as long as necessary to prevent the undesired condition. In such instances, maintenance of blood cholesterol level is often an objective as well as prevention of or reducing the risk of developing atherosclerotic disease or cardiovascular disorders such as heart attack and stroke.

#### Compositions of the present invention

The pharmaceutical compositions of the present invention comprise a pharmaceutically effective amount of an HMG-CoA reductase inhibitor and a pharmaceutically effective amount of a caspase inhibitor. These compositions are useful for treating or preventing elevated blood cholesterol in a mammal in need thereof while counteracting the potentially adverse effects, such as myopathy, that can be associated with the administration of the HMG-CoA reductase inhibitor.

#### HMG-CoA Reductase Inhibitor

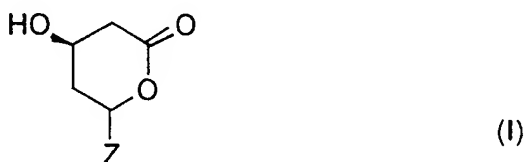
The compositions herein comprise a compound which inhibits the enzyme, HMG-CoA reductase. Compounds which have inhibitory activity for HMG-CoA reductase can be readily identified by using assays well-known in the art. See U.S. Patent No. 4,231,938, to Monaghan et al., issued November 4, 1980 and U.S. Patent No. 5,354,772, to Kathawal, issued October 11, 1994, both of which are incorporated by reference herein in their entirety.

Examples of HMG-CoA reductase inhibitors that are useful herein include but are not limited to lovastatin (MEVACOR®; see U.S. Patent No. 4,231,938, already cited above and incorporated by reference herein), simvastatin (ZOCOR®; see U.S. Patent No. 4,444,784, to Hoffman et al., issued April 24, 1984), pravastatin (PRAVACHOL®; see U.S. Patent No. 4,346,227, to Terahara et al., issued August 24, 1982), fluvastatin (LESCOL®; see U.S. Patent No. 5,354,772, already cited above and incorporated by reference herein), atorvastatin (LIPITOR®; see U.S. Patent No. 5,273,995, to Roth, issued December 28, 1993) and cerivastatin (also known as rivastatin; see U.S. Patent No. 5,177,080, to Angerbauer et al., issued January 5, 1993); and mevastatin (compactin, see U.S. Patent No. 3,983,140, to Endo et al, issued September 28, 1976. The patents cited in the previous sentence not already incorporated by reference are also incorporated by reference herein in their entirety. The structural formulas of these and additional HMG-CoA reductase



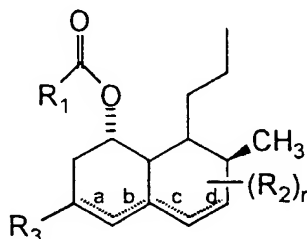
inhibitors that can be used in the present invention are described at page 87 of M. Yalpani, "Cholesterol Lowering Drugs", Chemistry & Industry, pp. 85-89 (5 February 1996), which is incorporated by reference herein in its entirety. The term HMG-CoA reductase inhibitor is intended to include all pharmaceutically acceptable lactone and open acid (that is where the lactone ring is opened to form the free acid), as well as salt and ester forms of compounds which have HMG-CoA reductase inhibitory activity, and therefor the use of such lactone, open acid, salt, and ester forms is included within the scope of this invention. Preferably, the HMG-CoA reductase inhibitor is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, mevastatin, and the pharmaceutically acceptable lactones, open acids, salts, and esters thereof, and mixtures thereof. More preferably, the HMG-CoA reductase inhibitor is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, and the pharmaceutically acceptable lactones, open acids, salts, and esters thereof, and mixtures thereof. More preferably, the HMG-CoA reductase inhibitor is selected from the group consisting of lovastatin, simvastatin, and the pharmaceutically acceptable lactones, open acids, salts, and esters thereof, and mixtures thereof.

Preferred HMG-CoA reductase inhibitors can be represented by the chemical formula



wherein Z is selected from the group consisting of:

a)



wherein  $R^1$  is  $C_1$ - $C_{10}$  alkyl,

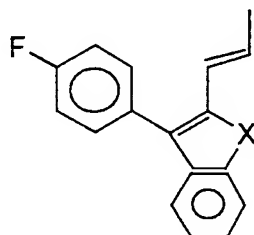
$R^2$  is selected from the group consisting of  $C_1$ - $C_3$  alkyl, hydroxy, oxo, and  $C_1$ - $C_3$  hydroxy substituted alkyl,

- 5  $R^3$  is selected from the group consisting of hydrogen, hydroxy,  $C_1$ - $C_3$  alkyl, and  $C_1$ - $C_3$  hydroxy substituted alkyl,

a, b, c, and d are all single bonds, or a and c are double bonds, or b and d are double bonds, or one of a, b, c, and d is a double bond, and

n is 0, 1, or 2;

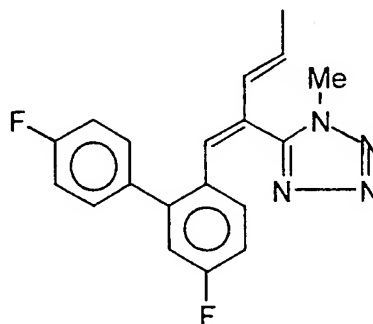
b)



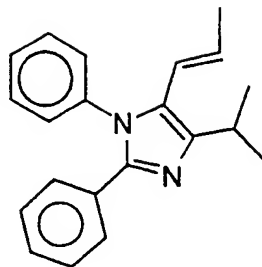
10

wherein X is selected from the group consisting of  $N[CH(CH_3)_2]$  and  $CH(CH_2)_3CH_3$

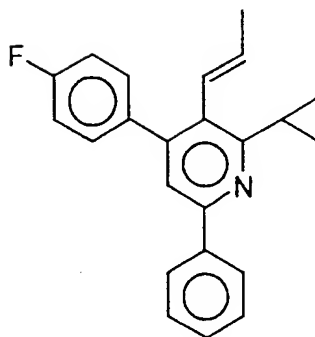
c)



d)



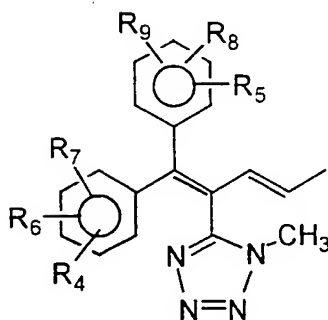
e)



and

5

f)



wherein R<sup>4</sup> and R<sup>5</sup> are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>1</sub>-C<sub>4</sub> alkoxy, and

trifluoromethyl, and R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, and R<sub>9</sub> are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, C<sub>1</sub>-C<sub>4</sub> alkyl, and C<sub>1</sub>-C<sub>4</sub> alkoxy. See U.S. Patent No. 5,650,523, to DeCamp et al., issued July 22, 1997, which is incorporated by reference herein in its entirety. The pharmaceutically acceptable lactone, open acid, salt, and ester forms of the compounds depicted by the preceding chemical formulas are intended to be within the scope of the present invention.

The term "pharmaceutically acceptable salts" as used herein in referring to the HMG-CoA reductase inhibitors shall mean non-toxic salts of the compounds employed in this invention which are generally prepared by reacting the free acid with a suitable organic or inorganic base. Examples of salt forms of HMG-CoA reductase inhibitors include, but are not limited to, acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynapthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, pamaote, palmitate, panthothenate, phosphate/diphosphate, polygalacturonate, potassium, salicylate, sodium, stearate, subacetate, succinate, tannate, tartrate, teoate, tosylate, triethiodide, valerate, and mixtures thereof.

The term "esters" as used herein in referring to the HMG-CoA reductase inhibitors is used in its standard meaning to denote the condensation product of a carboxylic acid and an alcohol. Ester derivatives of the described compounds can function as prodrugs which, when absorbed into the bloodstream of a warm-blooded animal, can cleave in such a manner as to release the drug form and permit the drug to afford improved therapeutic efficacy.

The term "lactones" is used herein in referring to the HMG-CoA reductase inhibitors is used in its standard meaning to denote a cyclic condensation product of a carboxylic acid and an alcohol, i.e. a cyclic ester.

The term "open acid" is used herein in referring to the HMG-CoA reductase inhibitors to denote that the lactone ring is open, i.e. uncyclized, to form the free acid.

It is recognized that mixtures of two or more HMG-CoA reductase inhibitors can be utilized.

The dosage regimen utilizing a HMG-CoA reductase inhibitor is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt or ester thereof employed. A consideration of these factors is well within the purview of the ordinarily skilled clinician for the purpose of determining the therapeutically effective or prophylactically effective dosage amounts needed to prevent, counter, or arrest the progress of the condition. The term "patient" includes mammals, especially humans. Administering of the drug or drugs to the patient includes both self-administration and administration to the patient by another person.

The precise dosage of the HMG-CoA reductase inhibitor will vary with the dosing schedule, the particular compound chosen, the age, size, sex and condition of the mammal or human, the nature and severity of the disorder to be treated, and other relevant medical and physical factors. Thus, a precise pharmaceutically effective amount cannot be specified in advance and can be readily determined by the caregiver or clinician. Appropriate amounts can be determined by routine experimentation from animal models and human clinical studies.

In particular, for daily dosing, the amounts of the HMG-CoA reductase inhibitor can be the same or similar to those amounts which are employed for anti-hypercholesterolemic treatment and which are described in the Physicians' Desk Reference (PDR), 52<sup>nd</sup> Ed. of the PDR, 1998 (Medical Economics Co), which is incorporated by reference herein in its entirety. For the additional active agents, the doses can be the same or similar to those amounts which are known in the art.

The HMG-CoA reductase inhibitors can be administered via a wide variety of routes including oral administration, intravenous administration, intranasal administration, injections, ocular administration, and the like.

A preferred route of delivery is oral administration.

Oral dosage amounts of the HMG-CoA reductase inhibitor are from about 1 to 200 mg/day, and more preferably from about 5 to 160 mg/day. However, dosage amounts will vary depending on the potency of the specific HMG-CoA reductase inhibitor used as well as other factors as noted above. An HMG-CoA reductase inhibitor which has sufficiently greater potency may be given in sub-

milligram daily dosages. The HMG-CoA reductase inhibitor may be administered from 1 to 4 times per day, and preferably once per day.

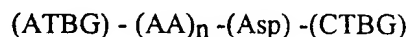
For example, the daily dosage amount for simvastatin can be selected from 5 mg, 10 mg, 20 mg, 40 mg, and 80 mg; for lovastatin, 10 mg, 20 mg, 40 mg and 80 mg; for fluvastatin sodium, 20 mg, 40 mg and 80 mg; for pravastatin sodium, 10 mg, 20 mg, and 40 mg; and for atorvastatin calcium, 10 mg, 20 mg, and 40 mg.

### Caspase Inhibitors

The compositions of the present invention comprise a pharmaceutically effective amount of a caspase inhibitor.

The caspase inhibitors useful herein are generally relatively short aspartic acid- containing peptides, although non-peptide inhibitors are also intended as being within the scope of the present invention. By "relatively short", as used herein means that the peptides typically contain from about 3 to about 5 amino acids in length. By "aspartic acid-containing" is meant that these peptides comprise at least one aspartic acid moiety, preferably at the carboxy-terminal end. These peptides are preferably blocked at both the amino and carboxy terminal ends with blocking groups.

The caspase inhibitors useful herein can be represented by the following chemical formula



Wherein (ATBG) is an amino terminal blocking group, (AA) is an amino acid moiety, "Asp" is aspartic acid moiety, (CTBG) is a carboxy terminal blocking group, and n is an integer from about 2 to about 4. In the caspase inhibitors, the amino acid (AA) can be selected from any of the naturally occurring amino acids, the D-enantiomers of the naturally-occurring amino acids (for example, D-alanine), and non-naturally occurring amino acids (for example, e.g., 3-aminopropionic acid and N-methyl glycine). The (ATBG) and (CTBG) moieties are selected from any of the blocking groups that are well known to peptide chemists of ordinary skill in the art. *See* Greene, T.W. et al., *Protecting Groups in Organic Synthesis*, 2nd edition, 1991, John Wiley & Sons, Inc., which is incorporated by reference herein in its entirety. Nonlimiting examples of (ATBG) moieties are benzyloxycarbonyl group (also known as the cbz or Z group) and the *t*-butoxycarbonyl group (also known as the boc group), and the acyl group. Nonlimiting examples of (CTBG) moieties are alkyl groups (for example methyl and

ethyl esters), the benzyl group, and the fluoromethyl keto group [which is abbreviated as (OMe)-CH<sub>2</sub>F or FMK].

Nonlimiting examples of caspase inhibitors useful herein are disclosed in U.S. Patent No. 5,210, 272, to Palmer, issued May 11, 1993, U.S. Patent  
5 5,101,068, to Palmer et al., issued March 31, 1992, and U.S. Patent No. 4,518,528, to Rasnick, issued May 21, 1985, which are all incorporated by reference herein in their entirety. Preferred caspase inhibitors useful herein are selected from the group consisting of Z-Val-Ala-Asp-FMK (which has a molecular weight of about 468), Z-Asp-Glu-Val-  
10 Ala-Asp-FMK (which has a molecular weight of about 630) MW 630. In the foregoing the standard three-letter amino acid abbreviations were used.

It is recognized that mixtures of two or more of the caspase inhibitors can be utilized.

The precise dosage of the caspase inhibitor will vary with the dosing  
15 schedule, the particular compound chosen, the age, size, sex and condition of the mammal or human, the nature and severity of the disorder to be treated, and other relevant medical and physical factors. Thus, a precise pharmaceutically effective amount cannot be specified in advance and can be readily determined by the caregiver or clinician. Appropriate amounts can be determined by routine experimentation from  
20 animal models and human clinical studies. Generally, an appropriate amount is chosen to counteract the potentially adverse myopathy effects of the HMG-CoA reductase inhibitor. The amount should be below that level which will inhibit the desired bone resorption inhibiting effect of the nitrogen-containing bisphosphonate. For humans, an effective oral dose of the caspase inhibitor is typically chosen so as to provide a local  
25 concentration in the esophagus from about 1  $\mu$ M to about 100  $\mu$ M, preferably about 10  $\mu$ M, although other ranges can be used. Nonlimiting exemplary doses are about 1 ug/kg to about 100 ug/kg, preferably about 10 ug/kg, for a human subject.

For the caspase inhibitor, human doses which can be administered are generally in the range of about 0.1 mg/day to about 10 mg/day, preferably from about  
30 0.25 mg/day to about 5 mg/day, and more preferably from about 0.5 mg/day to about 1.5 mg/day. A typical nonlimiting dosage amount would be about 0.75 mg/day. The pharmaceutical compositions herein comprise from about 0.1 mg to about 10 mg, preferably from about 0.25 mg to about 5 mg, and more preferably from about 0.5 mg to about 1.5 mg of the caspase inhibitor. A typical nonlimiting amount for is about  
35 0.75 mg.

#### Other components of the pharmaceutical compositions

The HMG-CoA reductase inhibitor and the caspase inhibitor are typically administered in admixture with suitable pharmaceutical diluents, excipients, or carriers, collectively referred to herein as "carrier materials", suitably selected with respect to oral administration, i.e. tablets, capsules, elixirs, syrups, powders, and the like, and consistent with conventional pharmaceutical practices. For example, for oral administration in the form of a tablet, capsule, or powder, the active ingredient can be combined with an oral, non-toxic, pharmaceutically acceptable inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol, croscarmellose sodium and the like; for oral administration in liquid form, e.g., elixirs and syrups, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated. Suitable binders can include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, and corn sweeteners, natural and synthetic gums, such as acacia, guar, tragacanth or sodium alginate, carboxymethyl cellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. The compounds used in the present method can also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropyl-methacrylamide, and the like.

#### Methods of the Present Invention

The present invention comprises methods for treating or preventing elevated blood cholesterol in mammals. In preferred embodiments of the present invention, the mammal is a human.

The compositions and methods of the present invention are administered and carried out until the desired therapeutic effect is achieved.

In the methods of the present invention the HMG-CoA reductase inhibitor and the caspase inhibitor are generally administered concurrently. In alternate embodiments, the HMG-CoA reductase inhibitor and the caspase inhibitor can be administered sequentially. Preferably, the caspase inhibitor is administered first.

The following Examples are presented to better illustrate the invention.



## EXAMPLE 1

Method for Evaluating the Effect of a HMG-CoA Reductase Inhibitor and a Caspase Inhibitor on Kinase Activities in Cultured Osteoclasts

- 5 Murine co-cultures of osteoblasts and marrow cells are prepared using the methods of Wesolowski, et al., *Exp Cell Res*, (1995), 219, pp. 679-686, which is incorporated by reference herein in its entirety. Bone marrow cells are harvested from 6-week-old male Balb/C mice by flushing marrow spaces of freshly isolated long bones (tibiae and femora) with  $\alpha$ -MEM (minimal essential media) containing
- 10 penicillin/streptomycin (100 I.U./ml of each and 20 mM Hepes buffer). The bone marrow cells are suspended in  $\alpha$ -MEM and the cells are filtered through an approximately 70  $\mu$ m cell strainer. The filtrate is centrifuged at about 300 x g for about 7 minutes. The resulting pellet is resuspended in  $\alpha$ -MEM supplemented with fetal calf serum (10 % v/v) and 10 nM 1, 25-(OH)<sub>2</sub> vitamin D<sub>3</sub>. These bone marrow
- 15 isolates are added to sub-confluent monolayers of osteoblastic MB 1.8 cells in cell culture plates and cultured for 7 days at 37°C in the presence of 5% CO<sub>2</sub>. Culture media is replenished every other day. Fusion of the osteoclast precursor cells from bone marrow (with each other) to form multinucleated osteoclast-like cells typically occurs after about 7 days. Osteoclast-like cells are enriched by sequential treatment with
- 20 collagenase (1 mg/mL in phosphate buffered saline) for one hour at 37°C and EDTA (0.2 g/L in phosphate buffered saline) for 20 min at 37°C. Non-adherent cells are rinsed away by washing with phosphate buffered saline. Osteoclast-like cells which are resistant to the sequential treatments are present at about 95% purity and are maintained in  $\alpha$ -MEM supplemented with fetal calf serum (10 % v/v), 10 nM 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub>, macrophage-colony-stimulating factor (5 ng/mL).
- 25

- The compounds to be evaluated are prepared as a solution of the desired concentration in  $\alpha$ -MEM. Examples of compounds that can be evaluated include HMG-CoA reductase inhibitors such as lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, mevastatin, and the pharmaceutically acceptable
- 30 salts, esters, and lactones thereof, as well as compounds that block the effects of these HMG-CoA reductase inhibitors, such as caspase inhibitors such as Z-Val-Ala-Asp-FMK, Z-Asp-Glu-Val-Asp-FMK, and Z-Tyr-Val-Ala-Asp-FMK. Combinations of compounds can also be evaluated. The solutions of the compounds to be evaluated are added to the cultures for a time period of 17-24 hours. No treatment controls

(controls not treated with compounds) are prepared by adding equivalent volumes of  $\alpha$ -MEM to the control dishes.

Cells are then harvested and lysed in a HEPES (N-(2-hydroxyethyl)piperazine-N'-(2-ethansulfonic acid) or Tris buffer containing the following:  $\beta$ -glycerophosphate (50 mM);  $\text{Na}_3\text{VO}_4$  (1mM); NaF (1mM); Microcystin LR (1  $\mu\text{M}$ ); leupeptin (10  $\mu\text{g}/\text{ml}$ ); aprotinin (10  $\mu\text{g}/\text{ml}$ ); phenylmethyl sulfonylfluoride (1 mM). Protein concentrations are determined for each lysate and 5-20  $\mu\text{g}$  are loaded into each lane of a SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) gel containing Myelin Basic Protein, or another kinase substrate, which has been polymerized into the gel at a concentration between 50-400  $\mu\text{g}/\text{ml}$ . Molecular weight standards are also loaded into one or more lanes of the gels. In-gel kinase assays are run according to a standard procedure based on Kameshita and Fujisawa, 1989 (Anal. Biochem. 183:139-143) and of Gotoh et al., 1990 (Eur. J. Biochem. 193: 661-669), both references being incorporated by reference herein in their entirety. The proteins are electrophoresed in the above gels. The gels are then successively soaked in 50 mM HEPES, pH 7.6; 5 mM 2-mercaptoethanol and each of the following (for each wash): (a) 20% isopropanol; (b) no additions; (c) urea (6 M); (d) Urea (3 M); (e) Urea (0.75 M); and Tween 20 (0.05% vol:vol). Kinase reactions are then run by first soaking the gels in 20 mM HEPES, pH 7.6; 20 mM  $\text{MgCl}_2$ ; 2 mM DTT and then in the same buffer containing 0.02 M ATP (non-radioactive) with ca. 1000 cpm/pmol  $^{32}\text{P}$ - $\gamma$ -ATP. The gels are then washed six times with 5% trichloroacetic acid and 1% pyrophosphate. The gels are then stained with Coomassie brilliant blue dye (0.125%) in 50% methanol, 10% acetic acid; destained with 30% methanol, 10% acetic acid; soaked in 2% glycerol; and dried using a gel dryer. The gels are then exposed to autoradiography film for times ranging from several hours to weeks. The bands observed in the autoradiographs representing the gels reflect kinase activities. Mst 1 (apparent molecular weight about 59 kDa), Mst 2 (apparent molecular weight about 60 kDa), and a 34 kDa Mst kinase fragment are observed and identified by their migration as compared to the migration of molecular weight standards. The band intensities on the autoradiography film are quantitated by densitometry and comparisons between bands from untreated controls and bands from echistatin-treated cells provide the basis for the analyses.

## EXAMPLE 2

Tablet composition

<u>Ingredient</u>	<u>Amount per tablet</u>
Simvastatin	10.0 mg
Z-Val-Ala-Asp-FMK	0.75 mg
BHA	0.02mg
Ascorbic acid	2.50 mg
Citric acid	1.25 mg
Microcrystalline cellulose	5.0 mg
Pregel starch	10.0 mg
Magnesium stearate	0.5 mg
Lactose	74.73 mg

All the ingredients except magnesium stearate are blended together in a suitable mixer. The powder mixture is then granulated with adequate quantities of granulating solvent(s), e.g. water. The wet granulated mass is dried in a suitable dryer. The dried granulation is sized through a suitable screen. The sized granulation is mixed with magnesium stearate before tableting. The tablets may be coated if deemed necessary. Additional ingredients that may be added to the above include suitable color and mixtures of colors.

The composition is useful for treating or preventing elevated blood cholesterol.

In alternative formulations, the simvastatin is replaced by a HMG-CoA reductase inhibitor selected from lovastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, or mevastatin, and the caspase inhibitor is replaced by Z-Asp-Glu-Val-Asp-FMK or Z-Tyr-Val- Ala-Asp-FMK.

## EXAMPLE 3

Directly compressed tablet composition

<u>Amount per tablet</u>	<u>Ingredient</u>
10 mg	Lovastatin
0.75 mg	Z-Val-Ala-Asp-FMK
116.9 mg	Microcrystalline cellulose
116.9 mg	Lactose anhydrate
7.5 mg	Crosmellose sodium
3.7 mg	Magnesium stearate

- 5                    The ingredients are combined and blended together and are compressed using conventional tableting techniques.

The composition is useful for treating or preventing elevated blood cholesterol.

- 10                   In alternative formulations, the simvastatin is replaced by a HMG-CoA reductase inhibitor selected from lovastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, or mevastatin, and the caspase inhibitor is replaced by Z-Asp-Glu-Val-Asp-FMK or Z-Tyr-Val- Ala-Asp-FMK.

## EXAMPLE 4

- 15    Hard gelatin capsule composition

<u>Amount per capsule</u>	<u>Ingredient</u>
10 mg	Simvastatin
0.75 mg	Z-Val-Ala-Asp-FMK
47 mg	Microcrystalline cellulose
47 mg	Lactose anhydrate
1 mg	Magnesium stearate
1 capsule	Hard gelatin capsule

The dry ingredients are combined and blended together and encapsulated in a gelatin coating using standard manufacturing techniques.

The composition is useful for treating or preventing elevated blood cholesterol.

In alternative formulations, the simvastatin is replaced by a HMG-CoA reductase inhibitor selected from lovastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, or mevastatin, and the caspase inhibitor is replaced by Z-Asp-Glu-Val-Asp-FMK or Z-Tyr-Val-Ala-Asp-FMK.

#### EXAMPLE 5

##### Oral suspension composition

10

<u>Amount per 5 mL dose</u>	<u>Ingredient</u>
10 mg	Lovastatin
0.75 mg	Z-Val-Ala-Asp-FMK
150 mg	Polyvinylpyrrolidone
2.5 mg	Poly oxyethylene sorbitan monolaurate
10 mg	Benzoic acid
to 5 mL with aqueous sorbitol solution (70%)	

An oral suspension is prepared by combining the ingredients using standard formulation techniques.

The composition is useful for treating or preventing elevated blood cholesterol.

15

In alternative formulations, the simvastatin is replaced by a HMG-CoA reductase inhibitor selected from lovastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, or mevastatin, and the caspase inhibitor is replaced by Z-Asp-Glu-Val-Asp-FMK or Z-Tyr-Val-Ala-Asp-FMK.

20

#### EXAMPLE 6

##### Intravenous infusion composition

<u>Amount per 200mL dose</u>	<u>Ingredient</u>
10 mg	Simvastatin

0.75 mg	Z-Val-Ala-Asp-FMK
0.2 mg	Polyethylene oxide 400
1.8 mg	Sodium chloride
to 200mL	Purified water

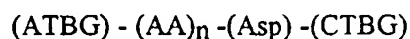
The ingredients are combined using standard formulation techniques.

The composition is useful for treating or preventing elevated blood cholesterol.

- 5           In alternative formulations, the simvastatin is replaced by a HMG-CoA reductase inhibitor selected from lovastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, or mevastatin, and the caspase inhibitor is replaced by Z-Asp-Glu-Val-Asp-FMK or Z-Tyr-Val- Ala-Asp-FMK.

## WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising an HMG-CoA reductase inhibitor and a caspase inhibitor.
2. A composition according to claim 1 wherein said HMG-CoA reductase inhibitor is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, mevastatin, and the pharmaceutically acceptable lactones, open acids, salts, and esters thereof, and mixtures thereof.
3. A composition according to claim 2 wherein said HMG-CoA reductase inhibitor is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, and the pharmaceutically acceptable lactones, open acids, salts, and esters thereof, and mixtures thereof.
4. A composition according to claim 3 wherein said HMG-CoA reductase inhibitor is selected from the group consisting of lovastatin, simvastatin, and the pharmaceutically acceptable lactones, open acids, salts, and esters thereof, and mixtures thereof.
5. A pharmaceutical composition according to any of Claims 1, 2, 3, or 4 wherein said caspase inhibitor is an aspartic acid-containing caspase inhibitor.
6. A pharmaceutical composition according to Claim 5 wherein said aspartic acid-containing caspase inhibitor corresponds to the following chemical formula



- wherein (ATBG) is an amino terminal blocking group selected from the group consisting of benzyloxycarbonyl, t-butoxycarbonyl, and acyl, (AA) is an amino acid, (Asp) is aspartic acid, (CTBG) is a carboxy terminal blocking group selected from the group consisting of C<sub>1</sub>-C<sub>6</sub> alkyl, benzyl, and fluoromethylketo, and n is an integer from about 2 to about 4.

7. A pharmaceutical composition according to Claim 5 wherein said aspartic acid-containing caspase inhibitor is selected from the group consisting of Z-Val-Ala-Asp-FMK, Z-Asp-Glu-Val-Asp-FMK, Z-Tyr-Val-Ala-Asp-FMK, and mixtures thereof.
- 5
8. A pharmaceutical composition according to Claim 7 wherein said caspase inhibitor is Z-Val-Ala-Asp-FMK.
9. A pharmaceutical composition which is prepared by combining
- 10 an HMG-CoA reductase inhibitor and a caspase inhibitor.
10. A method for treating or preventing elevated blood cholesterol in a mammal in need thereof comprising administering an HMG-CoA reductase inhibitor and a caspase inhibitor.
- 15
11. A method according to Claim 10 wherein said mammal is a human.
12. A method according to claim 11 wherein said HMG-CoA
- 20 reductase inhibitor is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, mevastatin, and the pharmaceutically acceptable lactones, open acids, salts, and esters thereof, and mixtures thereof.
- 25
13. A method according to claim 12 wherein said caspase inhibitor is selected from the group consisting of Z-Val-Ala-Asp-FMK, Z-Asp-Glu-Val-Asp-FMK, Z-Tyr-Val-Ala-Asp-FMK, and mixtures thereof.
- 30
14. A method for treating or preventing atherosclerosis in a mammal in need thereof comprising administering an HMG-CoA reductase inhibitor and a caspase inhibitor.
- 35
15. A method according to Claim 14 wherein said mammal is a human.



16. A method for treating or preventing cardiovascular disease in a mammal in need thereof comprising administering an HMG-CoA reductase inhibitor and a caspase inhibitor.

5

17. A method according to Claim 16 wherein said mammal is a human.

18. A method for treating or preventing a heart attack in a human in need thereof comprising administering an HMG-CoA reductase inhibitor and a caspase inhibitor.

10

19. A method for treating or preventing stroke in a human in need thereof comprising administering an HMG-CoA reductase inhibitor and a caspase inhibitor.

15

20. A method for treating or preventing elevated blood cholesterol in a mammal in need thereof comprising sequentially administering a caspase inhibitor compound and an HMG-CoA reductase inhibitor.

1/1

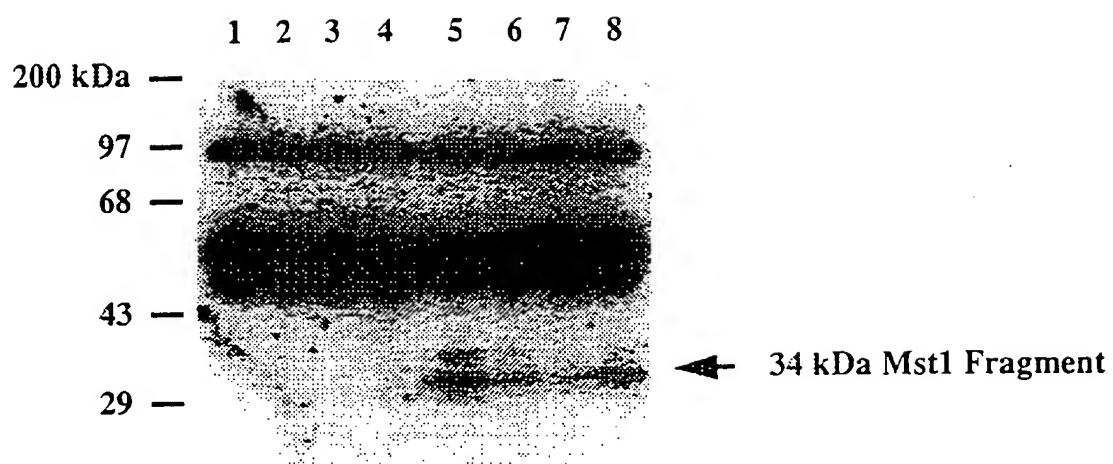


FIG.1

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/13888

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/405; 38/06, 38/07

US CL : 514/02, 18, 412, 415, 675

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/02, 18, 412, 415, 675

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN ON LINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KROON et al. LDL-cholesterol lowering and atherosclerosis - clinical benefits and possible mechanisms: an update. Netherlands Journal of Medicine. 1997, Vol. 51, pages 16-27.	1-20
A	Database CAPLUS on STN, AN 1997:713669. VILLA et al. 'Caspases and caspase inhibitors'. Trends Biochem. Sci., 1997, Vol. 22(10), pages 388-393.	1-20

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

16 SEPTEMBER 1999

Date of mailing of the international search report

20 OCT 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

MICHAEL BORIN

Telephone No. (703) 308-0196

JOYCE BRIDGERS  
PARALEGAL SPECIALIST  
CHEMICAL MATRIX

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/13888

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	Database BIOSIS on STN, AN 1999:112431. VITALE et al. 'Phenyltransferase inhibitors induce apoptosis in proliferating thyroid cells through a p53 independent, CrmA-sensitive, and caspase-3-like protease-dependent mechanism'. Endocrinology, February 1999, Vol. 140, No. 2, pages 698-704.	

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☒ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**